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DEPARTMENT OF THE ARMY

Fort Detrick

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**THE IMMUNOFLUORESCENCE TEST FOR TREPONEMA
APPLIED TO DRY BLOOD**

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The immunofluorescence test for Treponema, undertaken for the serological diagnosis of syphilis, may be carried out on total blood which is first absorbed on paper and then dried. This type of sampling makes it possible to send the blood by mail even to a very distant laboratory, and prevents the inconvenience of storage and transportation of blood or fresh serum. The excellent preservation of the immunological properties of the blood under these conditions, combined with the advantages of sensitivity and specificity of the immunofluorescence reaction, further increases the interest of this method.

*

The technique of immunofluorescence applied to the serodiagnosis of syphilis (FTA) occupies a position immediately after the Treponema immobilization test (TPI) among the most sensitive, most specific and most certain methods of the serodiagnosis of syphilis. It was developed by Deacon, Falcone and Harris in 1957 [1], and has since that time been studied by numerous serologists and treated in a large number of publications.

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The principle of this method consists in demonstrating the formation of the antigen-antibody complex by means of a fluorescent anti-gamma-globulin corresponding to the animal species of the serum to be examined. The antiglobulin will be fixed on the antibody, which, itself, is already fixed on the antigen (*Treponema smear*), and will thus make it fluorescent. In the absence of antibodies, the antiglobulin will not be fixed and the antigen will remain nonfluorescent.

This technique has been performed in our laboratory for several years, and we always compare it to the other serological reactions, both of the lipidic and the treponemal kind. In a previous note [2] we have reported all the technical details, as well as the first results obtained.

So far the FTA and the TPP remain methods which are not yet in general use at the majority of medical analytical laboratories, but they are very much in demand by clinicians and are carried out in large series in specialized centers. To be sure, the transportation of blood samples poses certain problems such as the preservation of the antibodies, the leaking of the blood, packing precautions which do not always prevent the breakage of tubes, microbial contamination, etc. These disadvantages are even more marked when we have to do with samples originating from warm countries (Africa, Asia and South America), from where the journey to the laboratory is generally a long and difficult one. For these countries, where the treponematoses are very widespread (venereal syphilis, yaws, bejel, endemic syphilis, pinta), the importance of specific reactions is even greater because here one encounters a larger number of falsely positive lipidic reactions (which have various causes) than in the temperate countries. To remedy these transportation difficulties and to facilitate the taking of blood samples, we have studied, at the instigation of the World Health Organization, the possibility of carrying out the FTA on samples of dried blood, obtained by puncturing the tip of the finger, thus avoiding venous puncture.

Of the various methods considered, the only one which we have retained consists in adsorbing the capillary blood on disks of blotting paper which, after being dried and placed in a plastic bag, may be sent in simple envelopes to the laboratories specializing in performing FTA's.

In the present work we shall report the results of the FTA's carried out on dry blood, compared with the FTA's usually carried out on serum.

TECHNIQUE

Preparation of Disks

Disks 15 mm in diameter are cut by means of a punch from Canson No 435 paper.

According to the Canson Company, this is a pure rag-type blotting paper weighing 0.25 kg per square meter, leaving no water-soluble substances. Ash content 1.5%.

Taking of Blood Sample

The blood may be taken by puncturing the tip of the finger and directly adsorbing it on several disks which must be totally impregnated. In this way one collects, on the average, 0.1539 g of total blood per disk, which corresponds to about 0.0846 g of plasma. The impregnated disks are left to dry in the open air for one to two hours, and when they are completely dry they are introduced into a small plastic bag in which they are stored until the time of reaction.

Preparation of the Reaction

A dry disk is taken up in 8 ml of buffer solution, pH 7.2, which corresponds to a 1:100 dilution of fresh plasma. This disk is allowed to soak for two hours, after which time the paper should be completely discolored and a reddish-brown eluate should be obtained. This eluate will serve directly for carrying out the immunofluorescence reaction, as if one had to do with a serum diluted to 1:100. The technique of FTA is then applied under exactly the same conditions as have been described previously [2, 3].

RESULTS

At the same time that blood samples were taken for the paper disks, samples were also taken by venous puncture, which permitted us to carry out comparative reactions. On the sera we have performed, in addition to the FTA, also a TPI, a Reiter B.-W. and a Kolmer B.-W. cardiolipin test, and two flocculation tests (Kline and Kahn). As for the disks, some were examined at the same time as the serum, while others were stored in plastic bags for a period ranging from a few days to six months.

So far we have examined in this manner 930 blood samples of different reactivities, at the first dilution

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examined (1:100). The results, compiled in condensed form in the following table, present a comparison between the FTA's on the serum and the FTA's on dry blood, carried out within 48 hours following the taking of the sample.

Sera	Disk of dry blood
95 samples +++	72 samples +++ 23 samples ++
187 samples ++	10 samples +++++ 162 samples +++ 15 samples ++
168 samples ++	38 samples +++ 124 samples ++ 6 samples +
180 samples +	30 samples ++ 102 samples + 27 samples +- 21 samples -
36 samples +-	12 samples + 19 samples +- 5 samples -
264 samples -	19 samples + 15 samples +- 230 samples -

This table shows that, compared with the results obtained with the serum, the results obtained with the disks were in perfect agreement in 709 cases, had a higher sensitivity in 124 cases and a lower sensitivity in 97 cases. However -- and this should be underlined -- these differences of sensitivity are very small, never exceeding plus or minus one cross. Such small differences are observed even when several examinations are carried out on the same serum. Moreover, the quantitative examinations which we have made on the serum and disks at the same time have furnished absolutely identical titers. Hence we can conclude that the FTA performed on disks of dry blood may, for practical purposes, be compared with regard to sensitivity and specificity to the titers carried out on the serum.

Preservation of the Reactivity of the Disks

As we have already pointed out, several disks were prepared from each blood sample: One of the disks was examined at the same time as the serum; the second was stored in a plastic bag at laboratory temperature (20-25°C) for periods ranging from eight days to six months; finally, the third disk of each sample was sent, in a simple plastic package, to countries having high temperatures (New Delhi, Brazzaville, Manila, etc.). The latter disks traveled for at least ten days in these various localities, where temperatures of up to 43°C have been recorded. On their return, these disks were examined simultaneously with those of the same blood samples which have been stored in our laboratory.

In none of the 120 samples which have been examined under these four conditions - fresh serum, fresh disks, disks stored in the laboratory, and disks which have traveled under the above-mentioned conditions - did we observe any decrease in reactivity; the differences observed were very small and never exceeded more than plus or minus one cross. These differences could be attributed more to the reproducibility of the technique than to the storage of the disks (sometimes the second examination was slightly more positive than the first, even after several months of storage).

We are continuing these storage tests, which will be discussed in a more detailed report to be published at a later date.

SUMMARY

↓ The immunofluorescence test, carried out on 930 samples of total dried blood absorbed on disks of Canson No 435 blotting paper, has furnished results that were identical with those obtained with the serum, in regard to both sensitivity and specificity.

The preservation of the reactivity of the disks of dry blood is perfect during a minimum period of six months at laboratory temperature (20-25°C) and even at higher temperatures (43°C).

The importance of this method resides mainly in the simplification of the method of taking the blood sample, which can be carried out without venous puncture, merely by puncturing the tip of the finger; and also in the ease of

transportation of the samples, which, placed in a plastic bag, can travel in the form of a simple letter, without the danger of breakage or hemolysis which exists when a tube of blood is being shipped. () ←

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